```
=> d his

     (FILE 'HOME' ENTERED AT 15:45:07 ON 02 JUL 2003)

     FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 15:45:21 ON
     02 JUL 2003
L1            64 S SYLVAN A?/AU
                 E SYLVAN A?/AU
L2            58 S E6 OR E2
L3             1 S L2 AND ELIDA
L4         75194 S PYROPHOSPHATE
L5         85663 S L4 OR PPI
L6          1032 S LUMINOMETRIC OR BIOLUMINOMETRIC
L7            75 S L6 AND L5
L8            22 S L7 AND (ALLELE OR SNP OR MUTATION OR VARIANT)
L9            11 DUP REM L8 (11 DUPLICATES REMOVED)
```

L9 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2003:334501 CAPLUS
DOCUMENT NUMBER: 138:363783
TITLE: Methods and kits for determining **allele** frequencies of genetic polymorphisms using primer extension
INVENTOR(S): Sylvan, Anna
PATENT ASSIGNEE(S): Swed.
SOURCE: U.S. Pat. Appl. Publ., 38 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003082566	A1	20030501	US 2002-85774	20020227

PRIORITY APPLN. INFO.: US 2001-271703P P 20010227
AB The present invention relates to a method of detg. the frequency of an **allele** in a population of nucleic acid mols., said method comprising pooling the nucleic acid mols. of said population, performing primer extension reactions using a primer which binds at a predetd. site located in said nucleic acid mols., and obtaining a pattern of nucleotide incorporation. Kits for detg. **allele** frequencies of genetic polymorphisms using primer extension are provided.

L9 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:800767 CAPLUS
DOCUMENT NUMBER: 137:289907
TITLE: Use of pyrophosphatase and apyrase to remove contaminants for improved single-nucleotide polymorphism analysis by pyrosequencing or BAMPER method
INVENTOR(S): Wakabayashi, Yuki; Kanbara, Hideki; Chou, Guo-Hua; Kamahori, Masao
PATENT ASSIGNEE(S): Hitachi Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 27 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002306180	A2	20021022	JP 2001-117232	20010416

PRIORITY APPLN. INFO.: JP 2001-117232 20010416
AB An improved method of single-nucleotide polymorphism (**SNP**) anal. by pyrosequencing or **bioluminometric** assay coupled with modified primer extension reactions (BAMPER) by eliminating **pyrophosphate** (**PPi**) with pyrophosphatase and deoxyadenosine 5'-triphosphate (dATP) with apyrase, is disclosed. A soln. contg. deoxyadenosine 5'-triphosphate .alpha.S-sulfate (dATP.alpha.S), deoxythiamine 5'-triphosphate (dTTP), deoxyguanosine 5'-triphosphate (dGTP), deoxycytidine 5'-triphosphate (dCTP), is treated with pyrophosphatase to remove **PPi**. Pyrophosphatase and apyrase may be immobilized on a solid support. Reagent kits, and app. for the method are claimed. In this sequencing method, the 4 different nucleotides are added stepwise to a DNA template hybridized to a primer in the presence of DNA polymerase, ATP sulfurylase, luciferase, and apyrase. When the added nucleotide is complementary to the DNA template, it is added to the growing DNA strand by DNA polymerase, releasing **pyrophosphate**. The

pyrophosphate reacts with APS (adenosine 5'-phosphosulfate) and ATP sulfurylase to form ATP which then induces luciferase to produce light. Unincorporated nucleotide and the ATP produced by sulfurylase are degraded between each nucleotide addn. step by apyrase. Finally, the amt. of the extension of the primers is detd. by reaction of APS with **pyrophosphate** released during the second step, and bioluminescent (luciferase) detn. of the resulting ATP.

L9 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:260068 CAPLUS
DOCUMENT NUMBER: 136:274231
TITLE: Quantitative detection of single nucleotide polymorphisms by a **bioluminometric** assay coupled with modified primer extension reactions (BAMPER)
INVENTOR(S): Kanbara, Hideki; Chou, Guo-Hua; Okano, Kazunobu; Kamahori, Masao
PATENT ASSIGNEE(S): Hitachi Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 24 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002101899	A2	20020409	JP 2000-300577	20000928
US 2003049628	A1	20030313	US 2001-945703	20010905

PRIORITY APPLN. INFO.: JP 2000-300577 A 20000928
AB A new method for **SNP** anal. based on the detection of **pyrophosphate** (**PPi**) is demonstrated, which is capable of detecting small **allele** frequency differences between two DNA pools for genetic assocn. studies other than **SNP** typing. The method is based on specific primer extension reactions coupled with **PPi** detection. As the specificity of the primer-directed extension is not enough for quant. **SNP** anal., artificial mismatched bases are introduced into the 3'-terminal regions of the specific primers as a way of improving the switching characteristics of the primer extension reactions. The best position in the primer for such artificial mismatched bases is the third position from the primer 3'-terminus. Contamination with endogenous **PPi**, which produces a large background signal level in **SNP** anal., was removed using PPase to degrade the **PPi** during the sample prepn. process. It is possible to accurately and quant. analyze **SNPs** using a set of primers that correspond to the wild-type and mutant DNA segments. The termini of these primers are at the **mutation** positions. Various types of **SNPs** were successfully analyzed. It was possible to very accurately det. **SNPs** with frequencies as low 0.02. It is very reproducible and the **allele** frequency difference can be detd. It is accurate enough to detect meaningful genetic differences among pooled DNA samples. The method is sensitive enough to detect 14 amol ssM13 DNA. The proposed method seems very promising in terms of realizing a cost-effective large-scale human genetic testing system.

L9 ANSWER 4 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2002:872773 SCISEARCH
THE GENUINE ARTICLE: 606RJ
TITLE: Escherichia coli single-stranded DNA-binding a molecular tool for improved sequence protein quality in pyrosequencing
AUTHOR: Ehn M; Ahmadian A; Nilsson P; Lundeberg J; Hober S (Reprint)
CORPORATE SOURCE: KTH, Dept Mol Biol, SCFAB, Roslagstullsbacken 21, S-10691

Stockholm, Sweden (Reprint); Royal Inst Technol KTH, Dept Biotechnol, S-10691 Stockholm, Sweden
COUNTRY OF AUTHOR: Sweden
SOURCE: ELECTROPHORESIS, (SEP 2002) Vol. 23, No. 19, pp. 3289-3299

Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61, D-69451 WEINHEIM, GERMANY.
ISSN: 0173-0835.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pyrosequencing is a four-enzyme **bioluminometric** DNA sequencing technique based on a DNA sequencing by synthesis principle. Currently, the technique is limited to analysis of short DNA sequences exemplified by single-nucleotide polymorphism analysis. In order to expand the field for pyrosequencing, the read length needs to be improved and efforts have been made to purify reaction components as well as add single-stranded DNA-binding protein (SSB) to the pyrosequencing reaction. In this study, we have performed a systematic effort to analyze the effects of SSB by comparing the pyrosequencing result of 103 independent complementary DNA (cDNA) clones. More detailed information about the cause of low quality sequences on templates with different characteristics was achieved by thorough analysis of the pyrograms. Also, real-time biosensor analysis was performed on individual cDNA clones for investigation of primer annealing and SSB binding on these templates. Results from these studies indicate that templates with high performance in pyrosequencing without SSB possess efficient primer annealing and low SSB affinity. Alternative strategies to improve the performance in pyrosequencing by increasing the primer-annealing efficiency have also been evaluated.

L9 ANSWER 5 OF 11 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001528635 MEDLINE
DOCUMENT NUMBER: 21459030 PubMed ID: 11574695
TITLE: Quantitative detection of single nucleotide polymorphisms for a pooled sample by a **bioluminometric** assay coupled with modified primer extension reactions (BAMPER).
AUTHOR: Zhou G; Kamahori M; Okano K; Chuan G; Harada K; Kambara H
CORPORATE SOURCE: Hitachi Ltd, Central Research Laboratory, 1-280 Higashi-Koigakubo, Kokubunji-shi, Tokyo 185-8601, Japan.
SOURCE: NUCLEIC ACIDS RESEARCH, (2001 Oct 1) 29 (19) E93.
Journal code: 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011001
Last Updated on STN: 20011029
Entered Medline: 20011025

AB A new method for **SNP** analysis based on the detection of **pyrophosphate** (**PPi**) is demonstrated, which is capable of detecting small **allele** frequency differences between two DNA pools for genetic association studies other than **SNP** typing. The method is based on specific primer extension reactions coupled with **PPi** detection. As the specificity of the primer-directed extension is not enough for quantitative **SNP** analysis, artificial mismatched bases are introduced into the 3'-terminal regions of the specific primers as a way of improving the switching characteristics of the primer extension reactions. The best position in the primer for such artificial mismatched bases is the third position from the primer 3'-terminus. Contamination with endogenous **PPi**, which produces a large background signal level in **SNP** analysis, was removed

using PPase to degrade the **PPi** during the sample preparation process. It is possible to accurately and quantitatively analyze **SNPs** using a set of primers that correspond to the wild-type and mutant DNA segments. The termini of these primers are at the **mutation** positions. Various types of **SNPs** were successfully analyzed. It was possible to very accurately determine **SNPs** with frequencies as low 0.02. It is very reproducible and the **allele** frequency difference can be determined. It is accurate enough to detect meaningful genetic differences among pooled DNA samples. The method is sensitive enough to detect 14 amol ssM13 DNA. The proposed method seems very promising in terms of realizing a cost-effective, large-scale human genetic testing system.

L9 ANSWER 6 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2001:837441 SCISEARCH
THE GENUINE ARTICLE: 480CC
TITLE: Quantitative detection of single nucleotide polymorphisms for a pooled sample by a **bioluminometric** assay coupled with modified primer extension reactions (BAMPER)
AUTHOR: Zhou G H; Kamahori M; Okano K; Chuan G; Harada K; Kambara H (Reprint)
CORPORATE SOURCE: Hitachi Ltd, Cent Res Lab, 1-280 Higashi Koigakubo, Kokubunji, Tokyo 1858601, Japan (Reprint); Hitachi Ltd, Cent Res Lab, Kokubunji, Tokyo 1858601, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: NUCLEIC ACIDS RESEARCH, (1 OCT 2001) Vol. 29, No. 19, pp. U33-U43.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0305-1048.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A now method for **SNP** analysis based on the detection of **pyrophosphate** (**PPI**) Is demonstrated, which is capable of detecting small **allele** frequency differences between two DNA pools for genetic association studies other than **SNP** typing. The method Is based on specific primer extension reactions coupled with **PPI** detection. As the specificity of the primer-directed extension Is not enough for quantitative **SNP** analysis, artificial mismatched bases are Introduced Into the 3 ' -terminal regions of the specific primers as a way of Improving the switching characteristics of the primer extension reactions. The best position In the primer for such artificial mismatched bases Is the third position from the primer 3 ' -terminus. Contamination with endogenous **PPI**, which produces a large background signal level In **SNP** analysis, was removed using PPase to degrade the PPl during the sample preparation process. It Is possible to accurately and quantitatively analyze **SNPs** using a set of primers that correspond to the wild-type and mutant DNA segments. The termini of these primers are at the **mutation** positions. Various types of **SNPs** were successfully analyzed. It was possible to very accurately determine **SNPs** with frequencies as low 0.02. It Is very reproducible and the **allele** frequency difference can be determined. it Is accurate enough to detect meaningful genetic differences among pooled DNA samples. The method Is sensitive enough to detect 14 amol ssM13 DNA. The proposed method seems very promising In terms of realizing a cost-effective, large-scale human genetic testing system.

L9 ANSWER 7 OF 11 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000493608 MEDLINE
DOCUMENT NUMBER: 20414763 PubMed ID: 10958643
TITLE: Determination of single-nucleotide polymorphisms by

real-time **pyrophosphate** DNA sequencing.
AUTHOR: Alderborn A; Kristofferson A; Hammerling U
CORPORATE SOURCE: Research & Development, Pyrosequencing AB, Uppsala, Sweden.
SOURCE: GENOME RESEARCH, (2000 Aug) 10 (8) 1249-58.
Journal code: 9518021. ISSN: 1088-9051.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001017

AB The characterization of naturally occurring variations in the human genome has evoked an immense interest during recent years. Variations known as biallelic Single-Nucleotide Polymorphisms (**SNPs**) have become increasingly popular markers in molecular genetics because of their wide application both in evolutionary relationship studies and in the identification of susceptibility to common diseases. We have addressed the issue of **SNP** genotype determination by investigating variations within the Renin-Angiotensin-Aldosterone System (RAAS) using pyrosequencing, a real-time **pyrophosphate** detection technology. The method is based on indirect **luminometric** quantification of the **pyrophosphate** that is released as a result of nucleotide incorporation onto an amplified template. The technical platform employed comprises a highly automated sequencing instrument that allows the analysis of 96 samples within 10 to 20 minutes. In addition to each studied polymorphic position, 5-10 downstream bases were sequenced for acquisition of reference signals. Evaluation of pyrogram data was accomplished by comparison of peak heights, which are proportional to the number of incorporated nucleotides. Analysis of the pyrograms that resulted from alternate allelic configurations for each addressed **SNP** revealed a highly discriminating pattern. Homozygous samples produced clear-cut single base peaks in the expected position, whereas heterozygous counterparts were characterized by distinct half-height peaks representing both allelic positions. Whenever any of the allelic bases of an **SNP** formed a homopolymer with adjacent bases, the nonallelic signal was added to those of the **SNP**. This feature did not, however, influence **SNP** readability. Furthermore, the multibase reading capacity of the described system provides extensive flexibility in regard to the positioning of sequencing primers and allows the determination of several closely located **SNPs** in a single run.

L9 ANSWER 8 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 2000:321545 SCISEARCH
THE GENUINE ARTICLE: 306PP
TITLE: Direct analysis of single-nucleotide polymorphism on double-stranded DNA by pyrosequencing
AUTHOR: Nordstrom T; Ronaghi M; Forsberg L; deFaire U; Morgenstern R; Nyren P (Reprint)
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM, SWEDEN (Reprint); ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM, SWEDEN; STANFORD UNIV, DNA SEQUENCING & TECHNOL CTR, PALO ALTO, CA 94304; KAROLINSKA INST, INST ENVIRONM MED, DIV BIOCHEM TOXICOL, SE-17177 STOCKHOLM, SWEDEN; KAROLINSKA INST, INST ENVIRONM MED, DIV CARDIOVASC EPIDEMIOL, SE-17177 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN; USA
SOURCE: BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (APR 2000) Vol. 31, Part 2, pp. 107-112.
Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND.
ISSN: 0885-4513.
DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 11
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pyrosequencing, a new method for DNA sequencing, is gaining widespread use for many different types of DNA analysis. The method takes advantage of four coupled enzymes in a single tube assay to monitor DNA synthesis in real time using a **luminometric** detection system. Here, we demonstrate the use of pyrosequencing for direct analysis of single-nucleotide polymorphism on double-stranded PCR product. Pyrosequencing data on the human glutathione peroxidase gene (GPXI) from several individuals were analysed and three different allelic **variants** were determined and confirmed. The possibility of further simplifying the sequencing and template-preparation steps is discussed.

L9 ANSWER 9 OF 11 SCISEARCH COPYRIGHT 2003 THOMSON ISI
ACCESSION NUMBER: 1999:296601 SCISEARCH
THE GENUINE ARTICLE: 185UL
TITLE: Real-time **bioluminometric** method for detection of nucleoside diphosphate kinase activity
AUTHOR: Karamohamed S; Nordstrom T; Nyren P (Reprint)
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM, SWEDEN (Reprint); ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: BIOTECHNIQUES, (APR 1999) Vol. 26, No. 4, pp. 728-&. Publisher: EATON PUBLISHING CO, 154 E. CENTRAL ST, NATICK, MA 01760. ISSN: 0736-6205.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 30
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A real-time, simple and sensitive method for detection of nucleoside diphosphate (NDP) kinase activity has been developed. The assay is based on detection of ATP generated in the NDP kinase reaction between a nucleoside triphosphate and adenosine diphosphate (ADP), by the firefly luciferase system In the presence of 0.3 mM dGTP, the K-m, for ADP was found to be approximately 30 mu M for the NDP kinase from Baker's yeast. In the presence of 250 mu M ADP: the K-m, for dATP alpha S dTTP alpha S, dGTP, dTTP, dCTP and GTP was found to be approximately 0.01, 0.03, 0.05, 0.25, 0.75 and 0.2 mM, respectively. The assay is sensitive and yields linear responses between 0.05-50 mU. The detection limit was found to be 0.05 mU of NDP kinase. The method was used to detect NDP kinase contamination in commercial enzyme preparations.

L9 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 1997:68048 CAPLUS
DOCUMENT NUMBER: 126:166933
TITLE: Detection of single-base changes using a **bioluminometric** primer extension assay
AUTHOR(S): Nyren, Pal; Karomohamed, Samer; Ronaghi, Mostafa
CORPORATE SOURCE: Dep. Biochemistry Biotechnology, Royal Inst. Technology, Stockholm, S-100 44, Swed.
SOURCE: Analytical Biochemistry (1997), 244(2), 367-373 CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A rapid **bioluminometric** technique for real-time detection of known single-base changes is presented. The concept relies on the measurement of the difference in primer extension efficiency by a DNA polymerase of a matched over a mismatched 3' terminal. The rate of the

DNA polymerase-catalyzed primer extension is measured by an enzymic **luminometric** inorg. **pyrophosphate** (**PPi**) detection assay (ELIDA) (P. Nyren (1987) Anal. Biochem. 167, 235-238). The **PPi** formed in the polymn. reaction is converted to ATP by ATP sulfurylase and the ATP prodn. is continuously monitored by the firefly luciferase. In the single-base detection assay, immobilized single-stranded DNA fragments are used as template. Two detection primers differing with one base at the 3' end are designed, one precisely complementary to the nonmutated DNA sequence and the other precisely complementary to the mutated DNA sequence. The primers are hybridized with the '-termini over the base of interest and the primer extension rates are, after incubation with DNA polymerase and deoxynucleotides, measured with the ELIDA. We show that the relative mismatch extension efficiency is strongly decreased by substituting the .alpha.-thiotriphosphate analog for the next correct natural deoxynucleotide after the 3'-mismatch termini. The possibility of using the technique for studies of mismatch extension kinetics for two polymerases lacking exonucleolytic activity is shown.

L9 ANSWER 11 OF 11 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 93167538 MEDLINE
DOCUMENT NUMBER: 93167538 PubMed ID: 8382019
TITLE: Solid phase DNA minisequencing by an enzymatic **luminometric** inorganic **pyrophosphate** detection assay.
AUTHOR: Nyren P; Pettersson B; Uhlen M
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 Jan) 208 (1) 171-5.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19980206
Entered Medline: 19930316

AB A solid phase DNA sequencing method for non-radioactive detection of single base changes without the need for electrophoresis is presented. The concept relies on the detection of DNA polymerase activity by an enzymatic **luminometric** inorganic **pyrophosphate** detection assay (P. Nyren, 1987, Anal. Biochem. 167, 235-238). Immobilized DNA fragments amplified with the polymerase chain reaction are used as template. A detection primer is annealed in front of the **mutation** and four aliquots of this mixture are incubated with DNA polymerase and one of the four different dideoxynucleotides. The presence or absence of an incorporated dideoxynucleotide is thereafter monitored by the release of inorganic **pyrophosphate** during the following primer extension step. We show that the concept can be used for sequencing of single bases as well as stepwise analysis of several subsequent bases.

L20 ANSWER 5 OF 24 MEDLINE

ACCESSION NUMBER: 2001087500 MEDLINE

DOCUMENT NUMBER: 21021219 PubMed ID: 11140947

TITLE: Cheap, accurate and rapid allele **frequency** estimation of single nucleotide polymorphisms by **primer extension** and DHPLC in DNA **pools**.

AUTHOR: Hoogendoorn B; Norton N; Kirov G; Williams N; Hamshere M L; Spurlock G; Austin J; Stephens M K; Buckland P R; Owen M J; O'Donovan M C

CORPORATE SOURCE: Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, United Kingdom.

SOURCE: HUMAN GENETICS, (2000 Nov) 107 (5) 488-93.
Journal code: 7613873. ISSN: 0340-6717.

PUB. COUNTRY: Germany: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200101

ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010118

AB At present, the cost of genotyping single nucleotide polymorphisms (SNPs) in large numbers of subjects poses a formidable problem for molecular genetic approaches to complex diseases. We have tested the possibility of using primer extension and denaturing high performance liquid chromatography to estimate allele frequencies of SNPs in pooled DNA samples. Our data show that this method should allow the accurate estimation of absolute allele frequencies in pooled samples of DNA and also of the difference in allele frequency between different pooled DNA samples. This technique therefore offers an efficient and cheap method for genotyping SNPs in large case-control and family-based association samples.

```
     (FILE 'HOME' ENTERED AT 13:21:36 ON 13 JUL 2003)

     FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 13:21:45 ON 13 JUL 2003
L1             3 S DETERMINING/TI AND SNP/TI AND FREQUENCIES/TI AND POOLS/TI
L2             1 DUP REM L1 (2 DUPLICATES REMOVED)

     FILE 'STNGUIDE' ENTERED AT 13:22:11 ON 13 JUL 2003
L3             0 S GENOTYPING/TI AND SNPS/TI AND OLIGONUCLEOTI/TI

     FILE 'MEDLINE, BIOSIS' ENTERED AT 13:23:30 ON 13 JUL 2003
L4             0 S GENOTYPING/TI AND SNPS/TI AND OLIGONUCLEOTI/TI
L5             3 S GENOTYPING/TI AND SNPS/TI AND OLIGONUCLEOTIDE/TI
L6             2 DUP REM L5 (1 DUPLICATE REMOVED)

     FILE 'STNGUIDE' ENTERED AT 13:24:04 ON 13 JUL 2003
L7             4 S POOL? OR POPULATION?

     FILE 'MEDLINE, BIOSIS, CAPLUS' ENTERED AT 13:25:53 ON 13 JUL 2003
L8       1782227 S POOL? OR POPULATION?
L9         68074 S (PRIMER EXTENSION) OR ARMS OR MICROSEQUENC? OR (SEQUENCING BY
L10         4209 S L8 AND L9
L11          962 S L10 AND FREQUENC?
L12          618 DUP REM L11 (344 DUPLICATES REMOVED)
L13            3 S L12 AND PYROPHOSPHATE
L14            3 DUP REM L13 (0 DUPLICATES REMOVED)
L15            0 S L12 AND APYRASE
L16            6 S L8 (5A) L9 (5A) (FREQUENC?)
L17            4 DUP REM L16 (2 DUPLICATES REMOVED)
L18           39 S L8 (10A) L9 (10A) (FREQUENC?)
L19           28 DUP REM L18 (11 DUPLICATES REMOVED)
L20           24 S L19 NOT L17

=>
```

L Number	Hits	Search Text	DB	Time stamp
1	1405995	snp or allele or variation or variant or mutation or mutant or polymorphism	USPAT; US-PGPUB; DERWENT	2003/07/02 15:08
2	23922	primer same exten$9	USPAT; US-PGPUB; DERWENT	2003/07/02 15:09
3	7329	allele same specific	USPAT; US-PGPUB; DERWENT	2003/07/02 15:09
4	27329	(primer same exten$9) or (allele same specific)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:18
5	30244	pyrophosphate or elida or ppi or luminometric or bioluminometric	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
6	142	(pyrophosphate or elida or ppi or luminometric or bioluminometric) same ((primer same exten$9) or (allele same specific))	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
7	1408892	pool$5 or population$ or individual$	USPAT; US-PGPUB; DERWENT	2003/07/02 15:11
8	11	((pyrophosphate or elida or ppi or luminometric or bioluminometric) same ((primer same exten$9) or (allele same specific))) same (pool$5 or population$ or individual$)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
9	30244	pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
10	142	(pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi) same ((primer same exten$9) or (allele same specific))	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
11	11	((pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi) same ((primer same exten$9) or (allele same specific))) same (pool$5 or population$ or individual$)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
12	0	(((pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi) same ((primer same exten$9) or (allele same specific))) same (pool$5 or population$ or individual$)) not (((pyrophosphate or elida or ppi or luminometric or bioluminometric) same ((primer same exten$9) or (allele same specific))) same (pool$5 or population$ or individual$))	USPAT; US-PGPUB; DERWENT	2003/07/02 15:17
13	838123	(primer same exten$9) or (allele same specific) or minisequenc$5 or arms	USPAT; US-PGPUB; DERWENT	2003/07/02 15:18
14	191	((primer same exten$9) or (allele same specific) or minisequenc$5 or arms) same (pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:19
15	11	(((primer same exten$9) or (allele same specific) or minisequenc$5 or arms) same (pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi)) same (pool$5 or population$ or individual$)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:19

17	0	((((primer same exten$9) or (allele same specific) or minisequenc$5 or arms) same (pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi)) same (pool$5 or population$ or individual$)) not (((pyrophosphate or elida or ppi or luminometric or bioluminometric) same ((primer same exten$9) or (allele same specific))) same (pool$5 or population$ or individual$))	USPAT; US-PGPUB; DERWENT	2003/07/02 15:20
18	719	(snp or allele or variation or variant or mutation or mutant or polymorphism) same (pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi)	USPAT; US-PGPUB; DERWENT	2003/07/02 15:20
19	147	((snp or allele or variation or variant or mutation or mutant or polymorphism) same (pyrophosphate or elida or ppi or luminometric or bioluminometric or ppi)) same primer	USPAT; US-PGPUB; DERWENT	2003/07/02 15:21